epartment of Agriculture Agricultural Research Service January 1990

# Agricultural Research



## Ph.D. Search Pays Dividends

## Food Safety: The Case for An Integrated View

One of the most interesting paradoxes in contemporary society is the widespread belief that the environment, in general, and the food supply, in particular, is unsafe and over the last century, has become increasingly unsafe. This belief is maintained even though, in this century, our society has seen a remarkable improvement in health.

In recent decades, revolutionary advances have forever altered the way scientists look at the living organism and its adaptation to its environment. One result of this process is greater uncertainty on the part of scientists in predicting the consequences of environmental change and on the part of the public in accepting the judgment of science. To some extent, the evolution of science itself is at the root of this belief.

In 1960, quantitative analysis at a level of 1 part in 1,000 was considered the limit of analytical chemistry. Today, technologies such as mass spectrometry, nuclear magnetic resonance, inductively coupled plasma techniques, and computer evaluation programs can routinely determine substances at 1 part in a billion and detect the presence of substances several orders of magnitude lower.

Because of this heightened capability, it's necessary to take a new look at the biological significance of such low numbers of molecules. Moreover, there's new evidence that many molecules which were believed exogenous to the environment are produced naturally, often at levels considerably higher than those attained by human use. Virtually all substances, natural or otherwise, contain low concentrations of compounds that would be toxic at higher levels.

The laws under which we operate evolved in an era when science did not require as much judgment to interpret as do for example, contemporary chemistry and biology. Thus the public has been told, on one hand, "there is no such thing as zero," and, on the other, "there is no such thing as absolute safety."

Without absolute scientific benchmarks on which regulatory decisions can be founded, the science underlying regulations may seem to the public largely a matter of judgment—not an arbiter of fact. One additional result of the evolutionary trend in safety assessment has been increasing interest in the process of estimating human risks with the hopes of reducing uncertainty and simplifying the evaluation.

Unfortunately, as we learn more, we find more questions to ask, increasing uncertainty even further and creating a need for even more sophisticated scientific evaluation.

We no longer view safety today as a simple dichotomous concept, nor is it absolute. Rather, it is a point on a continuum. Its exact position may be determined by several factors and may be influenced by social, political, legal, economic, and other issues.

Part of the problem in meeting this challenge is the difficulty of finding common units to express both risks and benefits. Almost invariably, benefits are expressed in economic terms while risks are expressed in more dramatic units such as lifespan or death or injury. It becomes difficult to compare the two without getting into the emotional issues of "how much is a human life worth?" Moreover, benefits are often multilayered and multidimensional. For example, a new process that results in substantial reduction in the cost of producing milk may have substantial health benefits by allowing more children to consume milk.

Nevertheless, risk evaluation has particular value in comparing the risks of various hazards or actions. Consider current fears concerning the presence of trace amounts of pesticides in foods. Any objective evaluation of the relative risk data would lead to the conclusion that, while continuing control of pesticide residues in foods is essential, current levels represent trivial risk as compared to other hazards.

Indeed, a comparison of the potential statistical risk associated with naturally occurring toxic substances in food is revealing. The risk associated with pesticide residues in food averages in the order of magnitude of $10^{-6}$. The risk associated with naturally occurring toxic substances in food, particularly carcinogens, is in the order of magnitude of $10^{-4}$ or $10^{-3}$. In contrast, the actuarial calculation of risk associated with microbiological contamination of foods reveals that the risk for morbidity, that is, the number of people who become ill, is in the order of $10^{-2}$ while the risk for mortality is $10^{-5}$.

The development of relative risk models leads to the consideration of risk distribution as a component of regulatory decisionmaking. As we become more attuned to the concept of risk distribution—the idea that risk reduction in one area may imply risk tolerance or neglect in another—we are gradually moving toward integrated safety policies which must explicitly take into account the entire spectrum of concerns and knowledge that affect food safety.

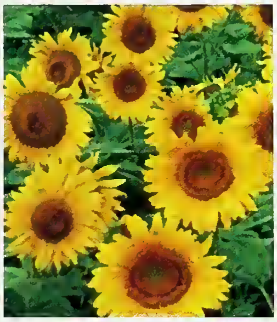
**Sanford A. Miller**

*This month's forum is based on the ninth Sterling B. Hendricks Memorial Lecture, delivered by Sanford A. Miller, September 11, 1989, before the American Chemical Society. Miller, now professor and dean at the Graduate School of Biomedical Sciences, University of Texas Health Science Center at San Antonio, was formerly director of the Center for Food Safety and Applied Nutrition at the Food and Drug Administration.*

# Agricultural Research

**Cover: Gloria Hoffman, one of many scientists to begin a career at ARS under the Postdoctoral Research Associate Program, examines bees at the Carl Hayden Bee Research Center, Tuscon, Arizona.** Photo by Jack Dykinga. (K-3438-1)





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# SEARCH FOR YOUNG SCIENTISTS PAYS DIVIDENDS

BARRY FITZGERALD

**Plant physiologist Grant Egley (background), laboratory director Steven Duke (middle) and Kevin Vaughn (foreground). (K-3352-9)**

January 1990 marks the 10th year that the Agricultural Research Service has searched the country for top-notch, recently graduated Ph.D's looking for a research challenge. And for ARS, it's a happy 10th anniversary.

Since 1980, more than 750 candidates have signed up for the 2-year ARS Postdoctoral Research Associate Program. And during the last 5 years, over 100 of them have earned employment as permanent, full-time ARS scientists.

Funded at $6.5 million, the 200 temporary positions offered this year can be the first step towards a life's career in science.

That's how it worked out for Kevin C. Vaughn, who was hired as a research associate in 1980 at the Southern Weed Science Laboratory in Stoneville, Mississippi. He was assigned to a research project that was already in progress.

"My first job was to study tentoxin, a fungal phytotoxin (substance poisonous to plants) that appeared to have potential as a natural herbicide," Vaughn says.

He found that tentoxin prevents the development of chloroplasts (where photosynthesis takes place) by limiting the uptake of an essential protein. Vaughn says tentoxin, a naturally occurring toxin of a fungal plant pathogen, will eventually kill a plant.

Since joining the lab as a permanent scientist in 1981, Vaughn has become an international authority on how weeds resist herbicides. He knows of about 300 cases in which weeds have developed a tolerance to currently used herbicides.

How did Vaughn come to ARS? Stephen O. Duke, current director of the Southern Weed Science Laboratory, recruited him.

"I myself had come to ARS 5 years earlier under a forerunner of the research associates program," Duke recollects.

At that time, the Southern Weed Science lab's Bonnie Reger and Grant Egley had issued a call for an eager young scientist willing to work hard at trying to determine weeds'

responses to light. Duke, who had just completed a dissertation on light control of the red pigment in plants, was hired.

A year later, his research clarified how the action of the phytochrome receptor site controls a weed seed's response to light. Phytochrome is the pigment in a seed that controls germination. Duke's research has been a model for subsequent work on using light to control seed germination.

Duke was appointed to permanent status to carry on Reger's work when she left the Weed Science lab. Since then, Duke has been named director of the lab and has become an authority on using natural products as herbicides and on determining modes of action of herbicides and other phytotoxins on weeds and crops.

Time passed. Following the trend that had worked so well, Vaughn hired research associate Alan R. Lax to continue the work on tentoxin. Lax discovered that a chloroplast envelope protein was associated with a plant's susceptibility to tentoxin.

"Once we isolated the protein, we realized that the envelope controlled the uptake. And when the chloroplasts can't get an adequate amount of protein, the plant turns yellow instead of a healthy green," Lax says.

When his tenure ended at Stoneville, Lax was asked by the Southern Regional Research Center to continue his tentoxin research on a permanent basis in New Orleans.

At SSRC, molecular biologist and research associate Jeffrey W. Cary is also broadening the tentoxin research challenge. "As a research associate, I'm working to clone the gene that encodes the protein affected by tentoxin," he says.

Successful cloning would provide large quantities of the pure protein so scientists could study how it interacts with tentoxin and how it is taken into the plant's chloroplasts.

### Vaccine Against Cattle Grub

At the Knipling-Bushland U.S. Livestock Insects Laboratory in Kerrville, Texas, ARS hired John H. Pruett to develop a vaccine against the cattle grub, *Hypoderma lineatum* and *H. bovis*.

The cattle grub could not be reared in the laboratory, so Pruett designed his research associate project to genetically engineer a vaccine. He identified pure antigens from cattle grub first-instar larvae that protected the cattle from the parasitic insect.

"From this research, we negotiated a cooperative technology transfer agreement with CODON, a San Francisco based company, to develop the vaccine," Pruett says.

The resulting patent, "Vaccines for the Protection of Animals Against Hypodermosis," (Serial No. 148,749) is held jointly by ARS and CODON.

Migrating larvae cause extensive damage to hides and meat. The larvae are currently controlled with chemicals, holding losses to about $60 million a year (1985). Pruett's vaccine would offer a nontoxic, environmentally safe future control.

Although hiring research associates has been successful from the agency's point of view, some postdocs have difficulty finding their own niche as a permanent scientist. Postdoc-turned-permanent ARS scientist Sue Mischke is familiar with the problem.

"Instead of just waiting around for their temporary research assignment to be changed into a permanent position or looking for the ideal position, I think postdocs must be adaptable. As the needs of ARS change, we must be able to change also," she says.

As a research associate at the Tissue Culture and Molecular Genetics Laboratory in Beltsville, Maryland, Mischke quickly learned to adapt. Her first ARS assignment was to develop electrofusion or electroporation techniques to transfer DNA into plant protoplasts.

At the time, cell fusion was done with a chemical; gene transfer was usually induced with a bacterium. Little was known about the effects of electric fields on living organisms.

Because specialized equipment was not commercially available, she and colleagues designed and constructed equipment needed to conduct experiments.

Electrofusion requires two electrical fields—an alternating current to group the protoplasts into "chains" and a direct current to break down cell membranes so they will fuse together. Electroporation, using direct electrical current to open pores of a membrane, allows materials to pass into the cell.

Mischke showed for the first time that protoplasts retain high viability after treatment by this technique. After examining movement of various molecules across plant cell membranes, she worked out conditions to fuse tobacco protoplasts.

Mischke is enthusiastic over the ARS postdoc program. The only drawback, she contends, is that many of the research projects tend to solve basic problems associated with initial

**Research associate entomologist Gloria Hoffman developing a computer-based pollination and nut set prediction model for almonds. (K-3439-2)**

JACK DYKINGA

research, which leads to few opportunities to publish. But if a researcher comes into a project that has been in progress for a couple of years, there's a better opportunity to accumulate publishable data.

Scientists hired under this program in other ARS locations are researching important issues and getting fascinating results:

- **Gloria Hoffman, Carl Hayden Bee Research Center, Tucson, AZ**—constructed a computer-based pollination and nut set prediction model for almonds that is currently being tested by California almond growers. As a permanent employee, Hoffman developed models to predict the genetic composition of a honey bee colony given the genotype of the queen and the drones she mated. This model allows simulation of the process by which a colony of European bees can become Africanized by the queen mating with Africanized drones.

  Hoffman found another line of parthenogenic honey bees that can raise queens and workers from eggs of laying workers. Until now only a honey bee from the Capetown region of South Africa showed a high frequency of this trait.

- **James A. Pfister, Poisonous Plant Research Laboratory, Logan, UT**—found that cattle can consume a sublethal dose of larkspur, a palatable but highly toxic range plant, without hurting their digestive system. Since consumption is regulated by gastrointestinal distress as well as plant flavor, animals can learn to reduce their intake. He defined a toxic "window" that predicts when most cattle die from a buildup of toxins.

  As a permanent scientist, Pfister now studies the relationship between the level of larkspur alkaloids—the toxic compounds—and consumption by cattle and sheep. He is also looking at feeding behavior, since ingesting a toxic compound may alter the way cattle feed.

- **Martin J. Shipitalo, North Appalachian Experimental Watershed, Coshocton, OH**—developed techniques to evaluate seasonal patterns of chemical movement affected by tillage. He also helped create a device to directly sample rainfall moving in earthworm burrows in the field. Shipitalo's research showed that earthworm holes are important pathways for water movement and that fertilizer nitrogen leaches more rapidly from no-till soils than those that have been tilled. Method of application, properties of chemicals used, and storm characteristics all affected the downward movement of chemicals.—By **Doris Sanchez,** ARS. ◆


DAN MILLER

**Range scientist James Pfister is studying ways to prevent or reduce larkspur poisoning in cattle (K-3443-1)**

**About the Research Associate Program**

ARS recruits about 160 postdoctoral research associates each year for positions throughout the country.

The positions are noncareer assignments that usually don't exceed 2 years. Applicants must have completed requirements for a Ph.D. degree before their employment. ARS prefers candidates with postdoctoral experience of no more than 3 years.

ARS usually hires research associates at the GS-11 grade level, starting salary of about $30,000. Costs for travel and moving household goods to the duty station can be covered in most cases. Health and life insurance benefits are available to associates appointed for more than 1 year.

For more information, telephone (301) 344-2749 or write to USDA, Agricultural Research Service, Personnel Management Branch, 6305 Ivy Lane, Greenbelt, MD 20770-1435. ◆

# ROOTING OUT GROUNDWATER CONTAMINATION

**Sunflowers roots help catch nutrients that may have escaped previous, shallower rooted crops. (K-3245-1)**

Sunflowers are sprouting up in more fields as farmers and the federal government become more creative in the business of making a living at the least expense to the environment.

It's all in a new crop rotation based on rooting depth. Sunflower and safflower, which reach down at least 6 feet, catch water or nutrients that may have escaped shallower rooted crops in the rotation.

Excess nutrients, especially nitrates from nitrogen fertilizer, can become toxic pollutants if they leach into the groundwater.

Al Black, director of ARS's Northern Great Plains Research Laboratory in Mandan, North Dakota, and colleagues set out to develop a crop rotation plan that minimizes the risk.

The rotation works like this: Spring wheat is planted about the first week of May. The crop is harvested in August. Then winter wheat is planted in September and harvested 10-1/2 to 11 months later, in July. Then in mid-May of the third year, sunflowers are planted. They are harvested in October and the next May the cycle begins again with planting of spring wheat.

In the Northern Great Plains, spring wheat roots extract water and nitrogen mostly from the upper 3 feet of soil and winter wheat, from the upper 4 feet.

Black says, "The roots of oilseed crops are like safety nets; they intercept any excess water and nitrogen before it can reach the groundwater."

The scientists tested this cropping pattern as part of a 63-acre study near Mandan. The site is part of a 400-acre farm for cooperative research by ARS and the Area IV Soil Conservation District.

The study includes the wheat-sunflower rotation, wheat-fallow, and each with various combinations of tillage and fertilizer applications.

In the new annual-crop rotation, spring wheat yields averaged 21.6 bushels per acre, winter wheat 29.7 bushels, and sunflowers 1,600 pounds an acre. Spring wheat on land that had been fallow averaged 31.2 bushels an acre, which amounts to 15.6 bushels annually.

Black admits that the new rotation goes against the traditional summer fallowing done in North Dakota and adjacent states. Farmers often let their land lie idle every second or third year to store up enough rainfall and snowmelt in the soil to raise a crop in the other years.

But, Black is quick to add, he is not calling for an end to summer fallowing. He says that's a necessary system for extreme drought years like 1988. He does advocate the annual rotation especially to avoid letting water get away during a series of relatively wet years.

Farmers need flexibility in their plans—to match crops to stored soil water, field by field, he says. He recommends testing soil moisture each spring before making planting decisions.—By **Don Comis,** ARS.

*Al Black is at the USDA-ARS Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554 (701) 663-6445.* ◆

# DIAGNOSING A STEALTHY STALKER

A subtle, mysterious disease, it attacks citrus-bearing trees of all ages. But it's most noticeable in young trees—at least 5 years old—that have just started producing fruit. It also attacks trees that are 20, 30, or even 50 years old.

There is no known cure. And, since the cause is unknown, there is no prevention.

"Even though it was first diagnosed in Florida in 1874, we know very little about citrus blight," says ARS plant physiologist Michael G. Bausher. "We just know that once it strikes, the tree becomes worthless."

But Bausher has just had a major research breakthrough that could be the first step in developing a rapid diagnostic test for the disease. Earlier detection could not only save trees, but also help in the search for cause and cure.

Citrus blight often starts on one side of the tree, gradually wilting leaves until it encompasses the tree. This wilt condition lasts throughout the remaining life of the tree. Partial tree defoliation and dieback of some branches usually occur. After symptoms are detected, 20-year-old trees may take from 1 to 3 years to die.

At the agency's U.S. Horticultural Research lab in Orlando, Florida, Bausher discovered unique proteins in leaves of citrus-blighted trees that aren't present in healthy trees.

"To be certain, these proteins were actually produced by blight and not by other types of disease stresses, we compared different stress source proteins," Bausher says.

He prepared, freeze-dried, and partially purified an antigen derived from ground-up leaves of blighted trees. Rabbits injected with the antigen produced antisera that reacted positively with blight-source proteins. Antisera responded negatively when exposed to proteins derived from healthy tissue, tissue infected with tristeza or *Phytophthora parasitica*, and water-stressed tissue.

RANDY SMITH

**Pale green, chlorotic leaves and reduced leaf size typical of citrus blight are displayed by plant physiologist Michael Bausher. (K-3323-3)**

According to Ronald Muraro, Florida loses about half a million citrus trees annually, valued at about $60 million, to citrus blight. Muraro, with the University of Florida's Citrus Research and Education Center (CREC) in Lake Alfred, says this estimate could be conservative.

Kenneth S. Derrick, also with the University of Florida's CREC, says that "growers usually pull trees from the orchard at the first sign of blight." Leaving them, he says, means smaller and less fruit the next season.

One of the difficulties in identifying the disease is that its symptoms can sometimes be mistaken for other disorders. Yellowing and mottling of leaves caused by insufficient zinc, a blight symptom, can also be caused by other diseases.

The other two diagnostic criteria associated with citrus blight are reduced water uptake and an accumulation of zinc in the wood of the tree. Leaves show a zinc deficiency while the wood has a higher zinc concentration than healthy trees.

Even though leaves of affected trees are wilted, the roots appear healthy—plentiful and full of starch. Despite the appearance, the roots do not take up water readily, so irrigation does not improve the tree's condition. This indicates a breakdown in the tree's transport system.

Growers now test for citrus blight by boring into tree trunks or removing root tissue to sample for zinc concentrations.

"Using the proteins as a biological marker should help us diagnose citrus blight before the symptoms appear," says Bausher.

He thinks early detection would help growers better decide whether to remove the tree from the grove at the first sign of disease . Even more important, it could help researchers develop plants that are resistant to citrus blight.

This, says citrus grower Orie Lee, would be nothing short of a miracle.

Lee runs a family-type operation in St. Cloud, about 25 miles southeast of Orlando. He grows just under 300 acres of oranges and grapefruit for processing. "From a grower's view-point, I consider citrus blight the number one problem of the citrus industry today," he says. "I've visited citrus growers in Brazil and my counterparts there also rate this disease their worst."

Lee says that in some of his groves, between 60 to 70 percent of the trees have been wiped out. Since so little is known about it, growers feel helpless and defenseless.

This is why he cooperates with University of Florida and ARS scientists at the Orlando lab who are looking for rootstock resistant to citrus blight.

Lee says even though there is no scientific data to back up the theory, most growers feel that when blighted trees are removed, the replantings are likely to be stricken at an earlier age than those removed.

"I've been in this business all my life," Lee says, "and have seen groves completely destroyed by blight. Any glimmer of hope that agricultural science can give us is more than we ever had before."

Bausher realizes the frustrations of long-term research with slow-incoming successes. So it doesn't discourage him that researchers have wrestled with the problem since the late 1800's with little success. In fact, citrus blight was the original reason for establishing a federally financed lab in Eustis, Florida, in 1892.

Bausher hopes his technique for identifying the proteins will arouse the interest of companies that develop commercial diagnostic kits.—**By Doris Sanchez,** ARS.

*Michael G. Bausher is at the USDA-ARS Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803 (407) 897-7353.* ◆

RANDY SMITH

**When citrus blight strikes, infected trees are destroyed and the immediate areas burned to remove dead tissue. (K-3323-4)**

### Citrus Lab Created To Study Blight

The U.S. Horticultural Research Laboratory was first established in 1892 at Eustis, Florida, about 35 miles northwest of its present location in Orlando. The lab's original purpose was to study citrus blight and improve the nation's citrus varieties.

Severe freezes in 1894-95 prompted scientists to begin research on cold hardiness as well. The lab was discontinued after another freeze in 1898-99, then reestablished at Orlando in 1935. The lab has about 70 employees, including 23 scientists, at its main site on Camden Road and two other Florida locations, the 500-acre A.H. Whitmore Foundation farm near Leesburg and a laboratory at Plymouth. ◆

# UNLOCKING THE HIDDEN ENERGY IN PLANTS

Lignin, which livestock can't digest, gives plants their structure and rigidity. It's the substance that cements cellulose and hemicellulose in and around plant cells like mortar and concrete hold bricks in a wall.

Because it ties up the energy in cellulose and hemicellulose, lignin is the most limiting factor in forage digestibility.

Many solutions to the problem of breaking lignin's bonds have been suggested . These range from traditional plant breeding and dowsing plants with a common household antiseptic and bleach to hitting crops with laser beams.

### Switching to Switchgrass

Forages supply more than 80 percent of all energy consumed by beef cattle; for dairy cattle, its 60 percent. Making forages more profitable is a key part of Kenneth P. Vogel's research at Lincoln, Nebraska.

Vogel, an ARS plant geneticist, worked with ARS geneticist Herman J. Gorz and University of Nebraska researcher Francis Haskins to give the Great Plains an improved switchgrass aptly named Trailblazer.

Switchgrass is a native of the American tallgrass prairie and thrives during the summer months when other grasses tend to grow slowly.

Feeding trials with Trailblazer showed that increasing digestibility of switchgrass by 6 percent boosted beef production 23 percent per acre. Over 50,000 acres have been planted to Trailblazer to date.

### Breaking Lignin's Bonds

In the mid-80's, chemist J. Michael Gould at the Northern Regional Research Center in Peoria, Illinois, began treating crop residues such as hay and corn stalks with hydrogen peroxide—a common household antiseptic and bleach. The effect was greater digestibility due to degradation of lignin by the bleach. This process has been refined and adapted for commercial use. [*Agricultural Research,* September 1989, p. 15]

ARS agronomist James R. Forwood is trying some high technology on lignin. He says microorganisms in an animal's rumen break down fiber faster if forage has had tiny holes punched in it by laser beams.

"Farmers now use grinding to break forage into more digestible pieces, and that takes a lot of costly energy. If someday lasers become as common as calculators are today, farmers may consider replacing grinders with lasers," says Forwood.

Previous work by Forwood in Columbia, Missouri, also showed that spraying plants with cellulase, a natural enzyme that breaks down cellulose in the animal's rumen, increased digestibility of tall fescue grass by 4 percent.

Using a new technique, scientists at the ARS Russell Research Center in Athens, Georgia, are getting more information about the chemical processes in a plant that result in limited digestibility.

ARS microbiologist Danny E. Akin and co-researchers developed the new technique using microspectrophotometry, which replaces less accurate analyses of whole plants or plant parts.

"The problem with analyzing whole plants is that some parts are digestible, some partially digestible, and some indigestible," says Akin.

Microspectrophotometry lets researchers look at individual cell walls and define digestibility. This analysis in part supports ARS breeding work in Tifton, Georgia.

Given specific information from Akin's analyses of various grasses, breeders can narrow down breeding criteria responsible for changing forage quality.

A new area of inquiry involving the study of anaerobic fungi living in the animal's rumen is being pioneered by the Athens group.

BRUCE FRITZ

**Plant geneticist Kenneth Vogel checks seed production of Trailblazer switchgrass. Vogel and cooperating ARS scientists recently found that the changes in digestibility appear to be genetically linked to a cell wall phenolic compound. (K-1787-7)**

DOUG WILSON

**Researchers may someday be able to prescribe certain grasses or supplements to cattle that would help maintain fungi that degrade lignancellulose in forages. (K-1223-12)**

The anaerobic fungi—so named because they live without oxygen—don't actually metabolize lignin; they attack tissues that contain lignin during digestion. The fungi produce enzymes that in turn release phenolic compounds.

If the anaerobic fungi were given an environment in which they could be more competitive with other bacteria living in the rumen, they could better do their job of weakening lignified tissue.

"Someday we may be able to recommend certain grasses or supplements to cattle diets that would encourage the growth of these fungi in the rumen," says Akin. He is looking for fungal species of this kind in other countries that are more active than those found in U.S. cattle.

Vincent H. Varel, ARS microbiologist at the Roman L. Hruska Meat Animal Research Center in Clay Center, Nebraska, has discovered an unusual bacterium, *Clostridium longisporum,* in a buffalo.

Varel's lab tests showed the microorganism can break down alfalfa feed 15 percent better than other rumen organisms. "Some studies indicate that buffalo can digest poor quality forages better than domestic cattle; however, it's not clear that this organism is responsible," says Varel.

Meanwhile, ARS researchers in St. Paul, Minnesota, are taking a different approach by making imitation plant cell walls to study the digestive process in a cow's rumen. Dairy scientist Hans G. Jung says the molecular structure of plant lignin plays an important role in limiting digestibility.

He is checking for genetic variation in fiber in smooth bromegrass and alfalfa. Tests are planned later for corn.

Besides plant genetics, Jung is looking at the fungi that cause wood to decay. Some of these fungi will selectively remove lignin, leaving behind a residue that is more digestible by rumen bacteria.—By **Linda Cooke,** ARS.

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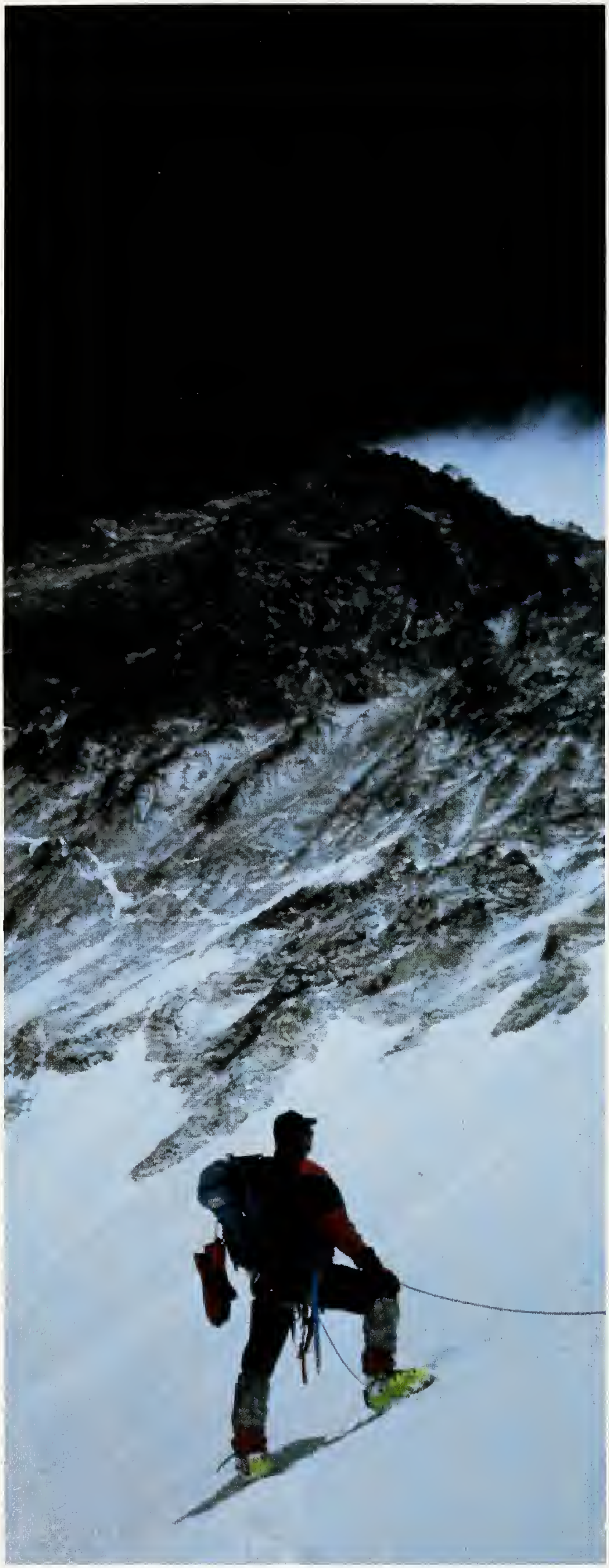

WALTER McCONNELL

# NUTRITION RESEARCH REACHES NEW HEIGHTS

*I took one step ... three gasps of air, one step ... three gasps of air. At one point, I tried to adjust my rhythm to one step ... two and a half gasps, so I could make two steps for every five gasps. But the body just doesn't seem to want to react to half gasps."*

*"I've run three marathons. I've never done anything more tiring in my life than this."*

*This is how ARS' Robert D. Reynolds describes climbing from 19,500 feet above sea level to 21,250 feet, carrying a 40-pound propane tank on his back. At these altitudes, a climber normally carries no more than about 18 pounds and climbs every other day, resting in between, he explains. "You're so exhausted that, unless it's an extreme emergency, you just cannot make carries 2 days in a row."*

*But this was an emergency. The second camp above base camp was out of propane to fuel the cook stove. With so little oxygen in the air and temperatures that plunge to -35°F at night, nutritious food can mean the difference between keeping or losing fingers and toes to frostbite. The climber's only source of heat is whatever his or her metabolism can generate.*

**With the summit of Mount Everest in the background, research chemist Robert Reynolds reaches a high point in his life at 22,600 feet along the Lhotse face.**

And the only source of drinking water is melted snow and ice. So Reynolds returned to Camp 1 for the needed propane the day after reaching his highest altitude, 22,600 feet.

Last spring, Reynolds, who normally specializes in vitamin $B_6$ studies at the Beltsville Human Nutrition Research Center, directed the first major nutrition study at extreme altitudes—above 15,000 feet—on the tallest mountain on Earth. He had been waiting for a decade for this opportunity: to learn the body's caloric needs under such extreme conditions and determine what types of foods or diets may improve performance and reduce physical and mental trauma.

Everest, says Reynolds, is ideal for such a study because its base camp—at 17,500 feet—is the highest in the world. And nonclimbers can hike to it. Similar studies have been done up to altitudes of 13,000 feet, he says. "You're stressing the body, but you're not getting that last point out on the curve. If you get the end point, you can interpolate back to various altitudes and exposures that people might experience."

Although Reynolds first got approval from ARS for such a study in 1980, he was dismayed to learn the mountain was booked decades in advance. In March of 1988, Walter McConnell, an emergency room physician from New Jersey and fellow climber, went to Nepal to assess the chances of getting a permit from the Ministry of Tourism. Fortuitously, word arrived that another group had cancelled for the spring of 1989 while McConnell was sitting in the minister's office.

With less than a year to raise private funds and gather supplies, he and Reynolds decided it was "now or never." Working almost round the clock, McConnell organized the fundraising and climbing schedule; Reynolds organized the research and assembled 11,000 pounds of food for the 9-week study.

Besides ARS funding which was limited to a portion of the nutrition research, public support came from the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, Maryland, the U.S. Navy, and the National Institute of Aging in Baltimore, Maryland.

Twelve men and two women made up the joint United States-Mexican expedition which left for Nepal in mid-February. Reynolds had left 3 weeks earlier to usher some 180 boxes of food and supplies through customs. On the mountain, the group was joined by 15 local residents, known as Sherpas, who would guide the climb or serve as cooks. Both of the Mexican men and three of the U.S. men were professional climbers; the other seven, including Reynolds, McConnell, and the two women were recreational climbers. One of the women was ARS biologist Mary Patricia (Pat) Howard, Reynold's technician at the Beltsville center.


FRED BOGNER

**Navy ophthalmologist Frank Butler (standing) conducts visual acuity tests on Robert Reynolds, who has just descended to base camp.**

Because most of the group was there for the climb, says Howard, "I was really surprised and pleased at how cooperative they were (in the study)." In addition to collecting a full day's urine output 27 times during the 9 weeks, they had to stick to certain diets and fill out two extensive questionnaires each day, noting their physical and mental symptoms, the quantity and type of foods they ate, those they craved, appetite level, and so on.

Since so little is known about the effect of extreme altitudes on nutrition, Reynolds explains, the team had to ask very fundamental questions. "But we tried to use the most sophisticated methods we could to get the most accurate and reliable answers." For instance:

- How many calories did the climbers consume?
- How many calories do people burn while climbing, or simply to maintain basic metabolism at extreme altitudes?
- Where is the weight loss, inevitable on such climbs, coming from—body fat, muscle, fluid, even bone?
- Can the climbers tolerate a high-fat diet? Does it give them a performance edge over a high-carbohydrate diet?

Howard remained at base camp during her 72 days on Everest to supervise urine analyses, skinfold measurements, blood work, and other tests in a makeshift laboratory. She had the help of two graduate students, Anneke Pietersma and Joyce de Stoppelaar, from the Agricultural University in Wageningen in the Netherlands.

Base camp sits on a carpet of rocks and rubble that avalanches have


**At the base camp, Dutch graduate student Anneke Pietersma performs a skinfold measurement on expedition leader Walter McConnell to check for changes in body fat and muscle composition.**

dislodged from higher walls. But directly below the rocks and rubble is a solid mass of ice.

"We were always cold in the morning," Howard says. Since there was no heat in the lab or anywhere else for that matter, "we just had to bundle up and wear gloves. But in the middle of the day, if it wasn't overcast or terribly windy, you could get down to a sweater." The lab's four walls of hand-piled rocks had many holes. "Wind would come right through and bring dust with it. If we set up for a blood draw the next day, we had to be sure we covered everything," says Howard, who isn't a stranger to research under adverse conditions. She had worked with Reynolds in 1983 on a nutrition study in the capital of Nepal.

Higher up, Reynolds and his Maryland neighbor, Kenneth E. Frick, recorded the climbers' food consumption and physical symptoms and handled the logistics of keeping the four high camps stocked with enough tents, sleeping bags, radios, batteries, stoves, and fuel. They also made sure the camps were supplied with high-fat or high-carbohydrate foods, according to the research protocol that day. Food for each camp was packed in a bag, and the climbers selected what they wanted and cooked it.

From a climber's standpoint, the expedition was a total success. On May 16, Ricardo Torres Nava enjoyed the thrill of becoming the first Mexican to reach the summit, along with two Sherpa guides.

From a researcher's standpoint, Reynolds expects to develop scientifically valid recommendations for food intake during extended exposure to high and extreme altitudes, as may occur during operations by the U.S. Armed Forces.

"It's doubtful that dietary changes would benefit people staying at high altitudes 1 or 2 days. But for the backpacker, climber, or skier, who may stay a week or two, we hope to recommend foods that enhance performance, or at least make the altitude a little easier to tolerate."

Although it will take another year to analyze samples and correlate all the data, he says preliminary findings have already exploded some myths. Most Everest expeditions report that their climbers lose about 30 percent of their starting body weight. This expedition averaged only a 10-percent loss.

And high-fat foods didn't cause nausea or precipitate the acute vomiting others have experienced at high altitudes. Nor did the climbers lose their taste for these foods. In fact, says Reynolds, at the higher camps, the climbers specifically requested some of the fattiest items—sausages, cheeses, and margarine.

Reynolds attributes these surprises to the fact that their food was so tasty. Before deciding on the rations for the expedition, Reynolds queried each of the climbers about their food preferences. Then he, Howard, and Sheri Henderson, rated every potential food item for palatability and ease of preparation before it was included in the menu.

"No matter how good a food is for you, if it tastes horrible, no one is going to eat it," says Reynolds. "And if it's fantastic to eat but very laborious to prepare, especially under these conditions, no one's going to take the time to prepare it."

The rations that passed muster were a big hit with climbers from other groups. They frequently swapped their own food or supplies, or "just happened to drop by our dining tent at mealtime every day," says Reynolds. Even the hikers, who often passed through base camp to become part of a major expedition for a few fleeting moments, were impressed. One American woman spotted an open package of Oreo cookies—a reminder of home that she had probably not tasted in months, he says. "She must have taken half a dozen pictures of it."

Biological technician Patricia Howard draws a blood sample from expedition member Timothy Thorne in the base camp makeshift laboratory.

ROBERT REYNOLDS

Even when the food is easy to prepare, the cooking can take hours. Reynolds describes a typical climbing day when food preparation began at 3:00 in the afternoon: "By the time you finished melting snow or ice to cook with, reconstituted the freeze-dried food and melted enough snow for drinking water for the next day it was 8:30 at night." Water may boil at a lower temperature way up in the clouds, but it takes a lot longer to get to that temperature. Much of the heat from a flame is lost before it reaches the bottom of the pan.

## High-Altitude Sickness

Two Navy ophthalmologists flew to Nepal to check the climbers' eyes for signs of bleeding when they descended to base camp. The Navy and Marines train special units for mountain warfare and want to know if the symptoms associated with high altitudes can be reduced.

Bleeding in the eye's retina is a common sign of high-altitude sickness, says Commander Frank K. Butler, with the U.S. Navy's Medical Corps, and it can cause some loss of vision. Other maladies, including acute swelling of the lungs and brain due to excess fluid, are problems that are probably a result of blood vessel damage due to the lack of oxygen, Butler says. But "we found significantly less hemorrhaging than we would have expected, based on reports" provided from other high-altitude climbs.

Since high-altitude climbers invariably lose weight, Reynolds wanted to know if any of that loss was coming from bone. "The National Institute of Aging is very interested in events that may lead to loss of bone minerals in the elderly," says Reynolds.

So 10 members of the expedition party donated blood samples and had bone scans at the institute in Baltimore, Maryland, before they left and after they returned.

Based on the bone scans, "we haven't found any significant changes," says Tracey Roy, a biologist with the Gerontology Research Center. Analysis of the blood and urine samples for calcium and hormone levels—not finished by this writing—could detect changes too small to show up on the scan.

Scientists at the Uniformed Services University jointly planned the diet regimen with Reynolds and Howard and provided part of the stable isotopes used to measure how many calories the climbers burned.

"They are very interested in the effects of cold and high altitude on energy expenditure, because military personnel could be exposed to such conditions," Reynolds explains.

The climbers alternated between a high-fat or high-carbohydrate diet every 3 weeks to see if either diet improved physical strength or reduced physical symptoms (see box). The only difference they noticed was more flatulence on the high-carbohydrate diet.

## Extremely Haute Cuisine

Reynolds says about a third of the food was what you would buy at a grocery store—crackers, dry soup mixes, cookies, candy bars, "and a lot of tortillas for our Mexican climbers." Another third was freeze-dried entrees purchased from outlets used by backpackers and hikers. These foods usually took longest to prepare since they had to be reconstituted with boiling water.

The final third was packaged, moist foods, purchased from a food manufacturer. Known as retort foods, these are precooked entrees or complete meals sealed germfree in a sturdy aluminum bag that will keep them preserved for months or years.

Several of the retort foods as well as the soups, a high-energy food bar, and some of the cookies and candy were among the favorites, Reynolds says. Retort entrees from Truitt Bros. of Salem, Oregon, were gobbled up and a retort sausage being developed by Oscar Mayer for the U.S. military

was "probably the highlight of the entire expedition."

He estimates that 30 percent of the food was purchased, usually at wholesale prices or cost. The rest was donated. Pepperidge Farms and Nabisco supplied cookies and crackers—"something you could really crunch into," says Reynolds. Hershey Chocolate provided candy bars; Provesta Corp. donated the popular high-energy food bar; and The Nestle Company provided hot chocolate mix. Campbell Soups donated dried soups, and Swanson supplied canned turkey and chicken.

Excluding research expenses, the expedition carried a price tag of $200,000, most of which was covered by corporate and private donations. Major donors were Agfa Films and Alka Seltzer Plus. Malaysian Airlines provided free round-trip transportation for the climbers and their cargo from Los Angeles to Bangkok, Thailand. And the Dutch Sugar Foundation sponsored the two graduate students.

There's another cost of an expedition to Everest. One-third of those who attempt to reach the summit in a given year are expected to perish; 1989 was a particularly costly year. Of the 14 climbers who reached the top, 7 died. And the joint American-Mexican expedition did not escape the toll. Phu Dorje, one of the two Sherpa guides who "summitted" with Torres Nava, lost his life on the way back down.—By **Judy McBride,** ARS.

*Robert D. Reynolds and M. Patricia Howard are at the USDA-ARS Beltsville Human Nutrition Research Center, BARC-East, Beltsville, MD 20705 (301) 344-2459.* ◆

**Everest—Outer Limits for the Human Body**

"You get higher and higher above the tree line, and there's just nothing there. It's like the end of the world," says graduate student Joyce de Stoppelaar.

It's also the outer limits for the body. Above 16,000 feet (base camp is at 17,500 feet) everybody stops breathing one or more times during sleep and wakes up gasping for air—terrified. These periods of sleep apnea get longer and more frequent as the climber ascends, explains climber and research leader Bob Reynolds.

Everybody also suffers from incessant bouts of heavy coughing—especially when resting or asleep—that can be violent enough to crack ribs and trigger the dry heaves. Reynolds says all members of this expedition complained of constantly running noses that were stopped up at the same time.

Above 20,000 feet, he continues, "the body is constantly deteriorating, losing weight. Metabolically, it becomes difficult to function." Some of the climbers developed fluid in the lungs—a major symptom of high-altitude sickness. But no one suffered swelling of the brain—not uncommon at these altitudes.

Whatever motivates a person to endure such adversity, the drive is almost overpowering. Fellow climber Kenneth E. Frick developed frostbite in one of his toes at about 21,000 feet. He spent a whole night debating, "Should I go up and lose the toe, or turn back and save it?" Prudence finally won out: Frick still has all 10 toes. And Reynolds forced himself to turn back at 22,600 feet after becoming dizzy and somewhat disoriented.

At 23,500 feet—where camp 3 is located—most climbers sleep with supplemental oxygen to generate enough metabolism to prevent frostbite in their hands and feet and a life-threatening drop in deep body temperature, says Reynolds.

At 26,500 feet—camp 4—the climber enters the "death zone" where the hardiest can't survive for more than a few days. Brain and body function slow dramatically. Appetite and thirst—the signal that the body is becoming dehydrated—virtually disappear, Reynolds says. "It's difficult to perform even routine functions like putting on your climbing boots or getting in and out of your sleeping bag." Only a handful of climbers have reached the summit without supplemental oxygen, he says, and they are metabolically exceptional.

Having been on the mountain, "I feel like a monkey has been lifted off my back," says Reynolds. "But I will not do Everest again—at all!"—By **Judy McBride,** ARS. ◆

# MICROBIAL COMBINATIONS FOR TOMORROW'S AGRICULTURE

Like friends who work together compatibly, some microbes can team up to help crop plants grow better.

Plant pathologist Robert G. Linderman and colleagues at the ARS Horticultural Crops Research Laboratory in Corvallis, Oregon, are pairing mycorrhizal fungi with other soil fungi and bacteria. When the fungi colonize the roots, they help the plant gather water and nutrients and help defend against root-devastating pathogens such as *Pythium, Rhizoctonia, Phytophthora,* and *Fusarium.*

Many of these microbes have evolved with each other and, according to Linderman, modern agriculture may have inadvertently disturbed these natural relationships, reducing the potential of soil organisms to help each other—and plants.

To compensate for the drop in the normal activity of the helpful microbes, growers use more fertilizers and more pesticides. But that can make things even worse for some microorganisms.

Linderman says, "That's why we're trying to develop technology growers can use to restore these carefully balanced underground ecosystems and find ways that powerful new combinations of microbes can be put to work for us."

So far, says Linderman, most of the biocontrol organisms tested, including helpful bacteria such as fluorescent pseudomonads and fungi such as *Trichoderma,* are compatible with mycorrhizae.

"Our experiments suggest that these combinations will improve disease suppression."

Linderman's research group has followed the lead of ARS plant physiologist Gabor J. Bethlenfalvay and his colleagues in Albany, California, in matching mycorrhizal fungi with beneficial bacteria that convert nitrogen from soil and air into a form plants can readily use.

Experiments by both groups indicate soybeans and other legumes will grow more vigorously if inoculated with the right strains of these two beneficial microbes as opposed to being inoculated with just one or the other. For example, at Corvallis scientists got up to 200 percent more growth of a leguminous crop treated with a rhizobium/mycorrhizal fungus combination.

"We still know little about how soil microbes affect one another," says Linderman, "but we've shown that finding the right blend of mycorrhizal fungi, rhizobia, and plant may be the key to improved yields."

Since mycorrhizal fungi aid plants, it's important to be sure that any other microbes growers might use—such as those that fight phytophthora root rot in the nursery, orchard, or field—don't injure the mycorrhizae.

Linderman and ARS colleagues Joyce E. Loper and Timothy C. Paulitz, along with Oregon State University cooperators Robert P. Griffiths and Bruce A. Caldwell, are developing easy-to-use tests for making sure that this doesn't happen.

The Environmental Protection Agency, responsible for evaluating proposed new microbial products, is funding part of this research.—By **Howard Sherman,** ARS.

*Robert G. Linderman is at the USDA-ARS Horticultural Crops Research Laboratory, 3420 Northwest Orchard Ave., Corvallis, OR 97330 (503) 757-4544.* ◆

**Through cooperative research, Oregon State University graduate student Roberto Valenciano and plant pathologist Robert Linderman evaluate the growth and health of poinsettias. The larger plant was inoculated with a combination of a mycorrhizal fungus and a biocontrol agent. (K-3329-10)**

MICHAEL THOMPSON

# BREEDING A BETTER PAPAYA


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A single papaya tree can produce as much as 300 pounds of fruit in just 8 months.

In Puerto Rico they call papaya the Mercedes crop.

"That's because if a grower can get just two or three good crops, he can probably afford to go out and buy a Mercedes. The fruit commands a premium price as an export, and a single tree can produce as much as 300 pounds of fruit," explains ARS scientist Paul Hepperly.

Hepperly, a plant pathologist at the ARS Tropical Crops and Germplasm Research Laboratory in Mayaguez, Puerto Rico, is seeking ways to improve the crop—to make it more cultivable and to better the papaya's flavor and texture.

The yellowish-green melonlike fruit has become increasingly popular in recent years with the U.S. crop up from 60,000 tons in 1985 to 69,000 in 1988 and imports of fresh papaya growing from 1,000 tons in in 1982 to 1,500 tons in 1988.

Papaya meat—which ranges from red to bright orange to light pink—can be sweeter than canteloupe, with a sugar content that may reach 17 percent. And the fruit can have over twice the vitamin A and C and more calcium than orange juice without any acidic taste.

The fruit is also a good source of a natural digestive enzyme called papain, used to clean contact lenses, to clear apple juice, and to tenderize meat. Medical applications are also being studied, according to Hepperly.

"With today's demand, if everything goes just right, a papaya grower with just 4 or 5 acres can bring in $100,000 a year," Hepperly says. "The problem is that the trees are very susceptible to several fungi, viruses, and mycoplasms. These pathogens seriously limit the crop."

Developing papayas with natural resistance to these diseases is one of Hepperly's major objectives. Success should reduce often costly and ineffective use of chemical insecticides and fungicides.

But papaya research is complicated by the extreme variability of offspring from each cross, Hepperly points out. In nature, papaya trees reproduce by cross pollination and each offspring may have a completely different combination of genes from the parents.

"So you get good trees and bad trees and some in between from each cross, which makes it very hard to develop a consistent variety," Hepperly says.

Careful hand pollination is required to begin developing a papaya that is more resistant to the papaya mosaic virus. This virus causes about a 40 percent drop in the rate of leaf photosynthesis in the plant, and dramatically decreases fruit sugar and taste.

Hepperly screened over 200 different trees from 5 populations—varieties can be so unstable that they are often simply called populations—to identify those with some natural resistance to viral, fungal, and bacterial disease.

The best of these populations typically has only about a 5 percent incidence of trees resistant to mosaic virus.

By intercrossing the most resistant individuals from the five populations, Hepperly has increased the incidence of resistance to about 50 percent of the population.

Hepperly is working on similar screening and crossbreeding searches for resistance to other diseases caused by mycoplasms, bacteria, and fungi.

"Some papaya researchers are looking to increase resistance by crossing species of papaya that would never cross in nature," Hepperly says. "So far, the offspring from these crosses do not produce good trees. I think the variation within varieties is sufficient that we can find enough resistance without resorting to this.

The large variability of papaya also applies to the fruit characteristics. Fruit can range from about 8 ounces and a basically round or pear shape to oval or club shaped and can weigh several pounds each.

The small pear-shaped ones are more popular in the United States, while Latin Americans prefer the larger, club-shaped fruit.

Hepperly is also concerned with improving taste and texture as well as size and shape. The lab benches are piled high with papaya fruit of every description that are carefully tested for sweetness, flesh consistency, and attractive appearance.

"We've got one cross between a Florida and a Puerto Rican variety that has better sugar content and firmer flesh as well as better yield than its parental varieties," Hepperly says. "The Florida variety was developed by ARS."

Research technician Ruth Ruberte and graduate assistant Jose Zamora work with Hepperly on the papaya evaluations.

To solve a common papaya problem of slow germination, they found that the watery envelope that surrounds a papaya seed needs to be removed.

The watery envelope contains a germination inhibitor.

By soaking and fermenting the seeds and then abrading and decanting the watery envelope, they have cut germination from 40 days to 10.

Genetic variation of offspring, the time it takes for tree development, and the fact that greenhouse disease-resistance results do not accurately predict field performance makes breeding in improvements slow work.

To speed things up, Hepperly has recently begun cooperating with several researchers at the University of Puerto Rico and the University of the Virgin Islands to try to apply tissue culture technology to papaya.

Tissue culture takes the cells of a plant from immature seeds, leaves, stems, or roots and regenerates them into whole plants. A single small sample can provide hundreds of genetically identical plants.

"If tissue culture techniques work with papaya, we will be able to increase the efficiency of breeding and shorten the time between generations," Hepperly says. "And we'll be able to have rapid replication of genetically identically plants. Then we'll be able to make progress much faster."—By **J. Kim Kaplan,** ARS.

*Paul R. Hepperly is at the USDA-ARS Tropical Crops and Germplasm Research Laboratory, P.O. Box 70, Mayaguez, PR 00709 (809) 831-3435.* ◆

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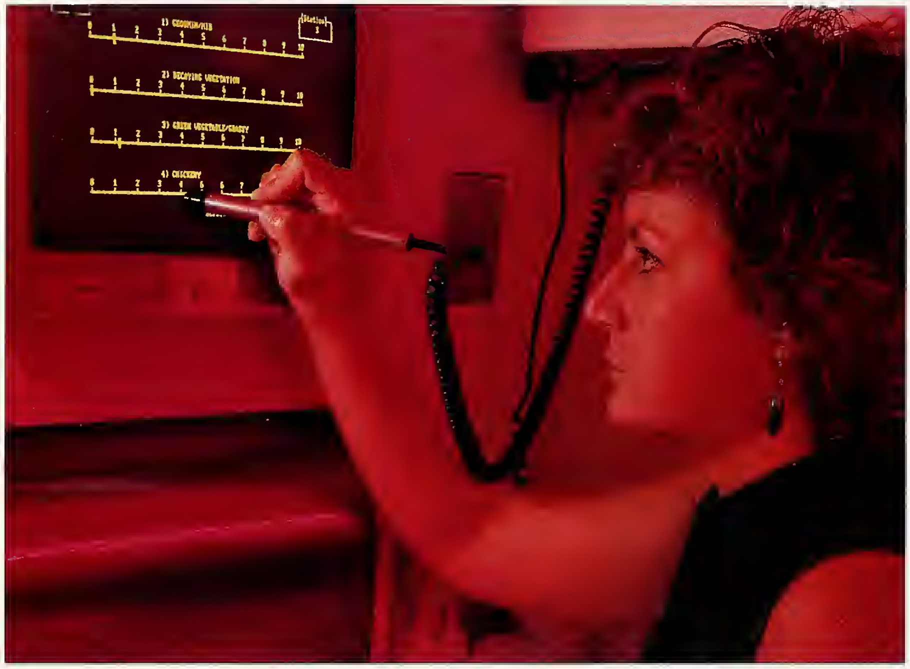
**Gene Bank Preserves Papayas**

America's official collection of papaya varieties from around the globe is in a gene bank on the Big Island of Hawaii, outside the town of Hilo and about 30 miles from Hawaii Volcanoes National Park. The National Clonal Germplasm Repository for Tropical and Subtropical Fruit and Nut Crops is a compound of modest buildings set in the Panaewa rainforest.

Curator Francis T.P. Zee says the collection, established in 1987, now has representive samples of most of the world's best-known papaya varieties. These range from Hawaii's red or yellow-orange commercial varieties, which Zee says taste better than those raised anywhere else, to cultivars grown in India for their prolific production of the enzyme papain.

Like other sites in the nationwide, ARS-managed gene bank system, the tropical crops collection is intended to preserve the genetic diversity of plant species and prevent the loss of valuable traits—such as insect and disease resistance—that may be carried in genes of wild and cultivated papaya. The collection is shared with papaya breeders worldwide.—By **Marcia Wood,** ARS.

*Francis T.P. Zee is at the USDA-ARS National Clonal Germplasm Repository for Tropical and Subtropical Fruit and Nut Crops, P.O. Box 4487, Hilo, Hawaii 96720 (808) 959-5833.* ◆



JACK DYKINGA

**In a taste panel booth, food technologist Carol Kelly rates intensity of individual flavors in catfish. (K-3440-9)**

## Taste Panel Ok's Catfish Feed

A recent study by U.S. Department of Agriculture and U.S. Fish and Wildlife Service scientists has found that as long as catfish feed contains about 40 percent protein, nearly any grain or animal byproduct can be used as an ingredient without spoiling catfish flavor, says Peter B. Johnsen of ARS' Food Flavor Quality Research Laboratory in New Orleans, Louisiana.

Johnsen, a research physiologist, says the findings prove that feed companies can safely retain current "least-cost feed formulation" practices.

In this method, a manufacturer substitutes ingredients as commodity prices fluctuate to maintain a predetermined nutritional level in the feed at the lowest cost.

Typical catfish feed contains 50 percent soybean meal, 25 percent corn, 10 percent fish meal. The rest is a mixture of vitamins, minerals, and binder.

Scientists tested feeds that contained wheat, corn, rice bran, cottonseed, grain sorghum, poultry byproducts, a variety of fish meals, and meat and bone meal. These experimental feeds were tested against a purified feed containing casein, a protein derived from milk. The casein-based diet was used as a benchmark to determine how feeds containing the different ingredients affect flavor quality.

Scientists checked flavor two ways: in the lab with a gas chromatograph/mass spectrometer that separates and identifies different aromas and in the tasting room where a highly trained human panel sampled fish from various feed regimens. The panel found very few differences.

The findings indicate that consumers can continue to expect high flavor quality, while the fish grower minimizes feed costs, Johnsen says.

In 1988, growers reaped $285 million in sales on about 295 million pounds of processed catfish, up 17 percent from 1987. Mississippi produces 85 percent of U.S. farm-raised catfish.—By **Bruce Kinzel**, ARS.

*Peter B. Johnsen is with the USDA-ARS Food Flavor Quality Research Laboratory, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70179 (504) 286-4421.* ◆

## Biocontrol Agent for Mushroom Flies

It's not enough for growers to keep mushrooms in the dark and feed them... compost. They may also need to supply them with a dose of Steinernematid nematodes.

Steinernematid nematodes, more formally descibed as *Steinernema bibionis,* may be the biocontrol weapon that the $500 million-a-year mushroom industry needs to fight the mushroom fly without resorting to insecticides, according to ARS scientists William R. Nickle and William Cantelo.

The tiny mushroom flies cut growers' profits by laying their eggs in the compost and then the hatched larvae eat the early stages of the mushrooms. They breed so quickly that their increasing population usually requires growers to use insecticides to prevent additional crop losses.

But Nickle and Cantelo have found adding Steinernematid nematodes, which are natural predators of flies, to mushroom growing medium can control the fly population.

The nematodes burrow into the compost and happily devour fly larvae.

"Nematodes are one of the few insect predators that like moist environments, which makes them useful biocontrol agents in this type of situation," says Nickle, who is a zoologist with the ARS Nematology Laboratory in Beltsville, Maryland. And since mushrooms like the same dark, moist conditions, the two are a very good match."

Nematodes wouldn't be found on the mushrooms themselves so consumers don't have to worry about them, Nickle adds.

"Right now, we can achieve 85 percent control of the flies by adding 600 Steinernematids per square centimeter of compost," Nickle says. (About 4,000 per square inch.) Even so, 85 percent is control only barely good enough for commercial growers to consider."

Nickle and Cantelo, an entomologist at the ARS Vegetable Laboratory

in Beltsville, have more tests planned to refine application rates and intervals in hopes of improving the control rate.

"It is possible that one day we will enable mushroom growers to get the full potential harvest without resorting to insecticides before the fly population swells to the point where growers have to start over again," Nickle says.

One reason Nickle and Cantelo see such potential in the nematode biocontrol idea is that Steinernematids are already commercially available.

"Biosys, a company based in California, sells Steinernematid nematodes for 10 to 20 cents per million," Nickle says. "And if they can establish a mushroom control market, the price could perhaps come down from that, making it economically attractive."

There is another possible benefit to the mushroom industry finding a better control method for the flies—better relations with their neighbors.

Many mushroom farms, located years ago in the country, are now surrounded by residential housing. These close-by suburbanite neighbors find mushroom flies to be bothersome pests.—By **J. Kim Kaplan,** ARS.

*William R. Nickle is at the USDA-ARS Nematology Laboratory (301) 344-3064. William Cantelo is at the USDA-ARS Vegetable Laboratory (301) 344-4557. Both are at the Beltsville Agricultural Research Center, Beltsville MD 20705.* ◆

## Rice Nurseries in Puerto Rico


BOB LLEWELLYN

**Rice plots in Puerto Rico. (K-3444-1)**

Twice each year, ARS agronomist Charles Bollich sends rice for a trip to the Caribbean island of Puerto Rico. These tropical trips are ARS' short cut to improving rice varieties.

Hidden away from the sandy beaches and sunny waters of Puerto Rico near the small town of Lajas, a tropical nursery cuts the time it takes to develop a new variety of rice by about one-third.

The climate and conditions in Lajas allow Bollich and his crew to harvest an additional two crops of rice a year, rather than rely on the single crop grown in Beaumont, Texas, the location of ARS' Rice Research Laboratory.

With the extra crops grown in Puerto Rico, Bollich was able to shave at least 3 years off the development of Lemont, now the leading rice variety grown in Texas and Louisiana and the second most popular variety in Mississippi. Lemont added over $3 billion to the economies of those three states between 1984 and 1988.

"Puerto Rico has basically the same growing conditions as Beaumont, but it has them all year long, so we can plant rice back right after we harvest it and really turn over two crops a year, in addition to one at Beaumont," Bollich says.

Something that Puerto Rico doesn't have is quarantine restrictions between it and the continental United States. Rice harvested from the nursery in Lajas can go directly back to Beaumont for further research.

"Not having to deal with restrictions on bringing in agricultural products, including rice, from Puerto Rico—restrictions we would face from almost any other island where growing conditions favor rice—is what makes this nursery work," Bollich explains.

The lack of restrictions doesn't mean the island harbors no rice diseases, just that the diseases are the same ones that are already in the United States.

The nursery was started in 1972 by Louisiana State University and the University of Puerto Rico, which then invited ARS to participate.

That first year Bollich planted 600 rows of rice. In the most recent season, more than 6,000 rice rows were planted.

The lushness of the climate actually creates a bit of a problem when it comes to weed control. "Even the weeds grow well here because there is no cold winter to kill them off," Bollich points out.

Included in many varieties that Bollich, one of the country's premier rice breeders, has developed for growers over the last 20 years is Rico I.

Rico I, a medium grain variety well adapted to Puerto Rico, was released by Bollich in support of an effort by Jose Vicente-Chandler, a now-retired ARS scientist, to develop a commercial rice industry on the island.

"It may take time to start up," Bollich says, "But someday the island will have a viable rice industry that will benefit the Puerto Rican people."

Some consumer preference and marketing tests were run at one time on the variety under the name Arroz D'Aqui, which means in Spanish "rice from here."—By **J. Kim Kaplan,** ARS.

*Charles Bollich is at the USDA-ARS Rice Research Laboratory, Route 7, Box 999, Beaumont, TX 77713 (409) 752-5521.* ◆

## Faster-Acting Menthol for Bees

Faster evaporation of menthol to kill tracheal mites, a major parasite of honey bees, now appears possible by mixing the chemical with vegetable shortening and spreading it on cardboard sheets.

In ARS tests, the menthol-shortening blend evaporated much faster than in earlier delivery systems: Twice as fast as loose menthol crystals in a screen packet, three times as fast as menthol pellets, and five times as fast as solid menthol cakes during the first 3 weeks of use. All forms of the menthol were inserted into hives and left in place for up to 10 weeks.

Speedy evaporation of the chemical is important to effectively control the mite, *Acarapis woodi,* a serious and growing problem for beekeepers across the United States, particularly in California, Washington, and New York. [See *Agricultural Research,* January 1988, p. 11]

The mite clogs the breathing tubes of adult bees, blocking oxygen flow and eventually killing the bees.

According to William T. Wilson, an entomologist at the ARS Honey Bee Research unit in Weslaco, Texas, the reason the menthol-shortening blend evaporates quickly may be tied to the uniform distribution of menthol molecules throughout the shortening.

Spreading the mixture in a thin film on a sheet of corrugated cardboard increases surface area, which may aid evaporation of the chemical.

"There may also be an interaction between the vegetable fat and menthol that transports the menthol to the surface of the film, where evaporation takes place," he says.

Besides vegetable shortening, the study included cardboard sheets with mixtures of menthol and petroleum jelly. Researchers found, however, that 50 grams of menthol added to 50 grams of shortening evaporated nearly four times faster than the same amount of menthol added to 50 grams of petroleum jelly.

Wilson cautions that more work needs to be done on the vaporization of menthol. While the chemical at correct treatment levels does not harm bees, he says, some bees were driven from their hives when field tests were conducted in hot weather. Menthol on the cardboard sheets evaporated faster than expected when temperatures climbed above 80 degrees, forcing the bees to evacuate and cluster outside the hives.—By **Matt Bosisio,** ARS.

*William T. Wilson is in the USDA-ARS Honey Bee Research Unit, Subtropical Agricultural Research Laboratory, P.O. Box 267, Weslaco, TX 78596 (512) 968-3159.* ◆

## Wasp Takes Whiff and Grain Pest Is History

The rusty grain beetle is not one to cover its tracks, even as the enemy draws near.

The larva of the beetle, while searching for a site in which to pupate, leaves a chemical trail that the females of *Cephalonomia waterstoni,* a parasitic wasp, can easily detect and follow.

The wasp is so sensitive to the chemical scent that she can follow a trail containing only one-thousandth of the amount of chemical present on the larva. And the trail persists in stored grain for up to a week.

"The response of the wasps was strikingly like that displayed by ants and termites engaged in trail following," says Ralph W. Howard, an ARS chemist at the Biological Research Unit in Manhattan, Kansas. "After they contacted the trail on filter paper in our tests, they proceeded to follow it in a zigzag manner, constantly drumming the paper with their antennae."

Occasionally, the wasps left the trail to briefly explore nearby areas. But they always returned to the trail to follow the scent, though sometimes in the opposite direction.

According to Howard, experience was a factor in how well the wasp followed the trail in laboratory tests. Wasps that had encountered rusty grain beetles earlier in their lives were more adept at trail following than were inexperienced wasps. The stronger the chemical scent, however, the better all wasps performed.

"This dosage-dependent searching behavior suggests that experienced wasps may indeed be able to assess the likelihood of finding a suitable host from the quantity of the chemical left on the grain," Howard says. " Naive wasps are innately sensitive to the chemical but their ability to efficiently follow the trail is undeveloped."

While *C. waterstoni* has been known to parasitize a few other insects, the rusty grain beetle is its primary target. The wasp uses the larvae of the beetle, a major pest of stored grain, as a host for its eggs.

The ultimate objective of the research, done jointly with ARS biologist Paul W. Flinn, is to keep the wasp active in stored grain for longer periods as a biological control of the beetle. "The goal is to understand the biology of the host-parasite relationship so the effectiveness of the parasite can be enhanced by changing grain management procedures," Howard says.—By **Matt Bosisio,** ARS.

*Ralph W. Howard and Paul W. Flinn are with the USDA-ARS Biological Research Unit, U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502 (913) 776-2706.* ◆

## Correction

**In "Weight Loss Is Simple Arithmetic," apppearing in the November 1989 issue of *Agricultural Research,* a caption misidentified biomedical engineer Jim Seale (right) and physiologist William Rumpler (left).**

## Rice Seed Dunked in Growth Regulator

The semidwarf rice varieties favored by farmers are more likely to deliver on their yield promises if their seed is treated with growth regulator, an ARS geneticist and University of Arkansas agronomist have discovered.

Semidwarfs such as Lemont and Gulfmont have become many farmers' first pick because they are less likely to lodge, dumping grain on the ground. In Arkansas, the nation's top rice-producing state, Lemont grows on 1 in 5 rice acres.

Still, these varieties are not without problems. Most notable is the narrow margin for error in planting depth.

Robert H. Dilday of ARS' Rice Production and Weed Control Research unit at Stuttgart, Arkansas, explains. "The mesocotyl and coleoptile elongate from the seed at germination. When the coleoptile breaks through the soil and sunlight hits it, you start getting leaf growth. But if the seed is too deep, the coleoptile never makes it to the soil surface."

Planting even a quarter inch too deep can result in a semidwarf stand that emerges unevenly or never shows up at all.

"In rice, everything you do is centered on the time of emergence of the stand," Dilday says. "If emergence is spread out over 2 to 3 weeks, you don't know when to time your fertilizer or herbicide applications."

Seeking a way to give semidwarf varieties a little extra push to emergence, Dilday and Ronnie S. Helms of the University of Arkansas hit on treating the seed with GA3, a type of gibberellic acid. Rice seed was treated in GA3 at 10, 50 or 100 parts per million and dried, and then planted.

"What we saw was a semidwarf that came up looking like some of the tall varieties," Dilday recalls. "At first, we thought we'd eliminated the semidwarf characteristics. In some instances, the length of the mesocotyl and coleoptile actually doubled.

"But in reality, the seedling just grows faster until it's 6 to 8 inches tall, then it loses the effect of the gibberellic acid. At maturity, there are no significant differences in plant height between treated and untreated semidwarf varieties, regardless of the rate of GA3 used.

Dilday and Helms tested the treatment on Lemont rice in growth chambers at Stuttgart and in fields at Stuttgart and Rohwer, Arkansas, in 1987 and 1988. Treated Gulfmont seed was also tested last summer by Abbott Laboratories in a 5-acre plot on a farm near Mayflower, Arkansas, with good results. The firm recently received a permit from the U.S. Environmental Protection Agency to field-test the promising treatment. During the 1990 growing season, 1,700 acres will be experimentally planted.—By **Sandy Miller Hays**, ARS.

*Robert H. Dilday is in Rice Production and Weed Control Research, P.O. Box 287, Stuttgart, AR 72160 (501) 673-2661.* ◆

### Patents

## Probe Pinpoints Powerful Hormones

If you take a leaf that's turning yellow and dying and put a drop of a compound known as cytokinin on it, in just a few days the spot where the drop hit will turn green. Little chloroplast energy factories within the leaf would have started back to work.

That's how powerful the cytokinin hormones of green plants are at thwarting natural aging.

And that's why plant breeders and molecular biologists dream of the day when they can manipulate the way plants handle cytokinins.

Revving up cytokinin output, for example, might stave off natural aging just long enough for crops like wheat to stash extra protein into each plump kernel before harvest. The opposite approach could also offer exciting new options: Slowing down a plant's normal cytokinin production could accelerate ripening. That might give growers a chance to squeeze two crops into a growing season that would normally yield only one.

Scientists are not talking about dousing crops with cytokinin-based sprays. Rather, these strategies rely on redirecting the plant's natural cytokinin production. To do this, however, scientists must first find answers to puzzling questions about when, where, and how plants make the hormone.

ARS scientists David L. Brandon and Joseph W. Corse of the Western Regional Research Center, Albany, California, have developed a new test that uses proteins called monoclonal antibodies to quickly find and snare cytokinins.

The probe seeks and binds to the hormone's inactive, or O-glycosylated form. Plants supposedly put that form into short-term storage, taking it out when they need to convert it back to the active chemical.

Plants could possibly be genetically programmed to put more of their cytokinin into the storage form and thus age faster. They might also be programmed to take more of it out of storage and fend off old age.

The probe is extremely sensitive. In small samples made from ground-up extracts of plant leaf, stem, or root, the new antibodies ferret out the hormone in quantities tinier than a nanogram (300,000 times smaller than a grain of salt).—By **Marcia Wood,** ARS.

*Technical information is available from David L. Brandon and Joseph W. Corse, USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710 (415) 559-5783. U.S. Patent Application Serial No. 07/334,069,* "Antibodies to Cytokinins Having a Glycosylated Isoprenoid Side Chain and Immunoassay Methods." ◆

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